Probing the Photocycle of Bacteriorhodopsin Microcrystals by FTIR Spectroscopy

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INTRODUCTION

Bacteriorhodopsin (bR) is a membrane protein that carries a covalently attached retinal group as a chromophore. Visible light (absorbance max = 568nm) captured by the chromophore provides bR with energy that is ultimately used to pump hydrogen ions out of the cell. The resulting proton-motive force powers ATP synthesis and drives the active transport of ions and nutrients across the cell.

Bacteriorhodopsin undergoes a series of chemical and structural changes as it transforms light energy into chemical energy. The photocycle of bR has been well characterized by both visible and IR spectroscopy. The most important intermediates are identified as K, L, M, N, and O; each has a distinct visible color and a distinct IR spectrum. The photocycle begins with the isomerization of all-trans retinal to 13-cis, followed by deprotonation of the Schiff base that links the protein to the retinal. A series of changes in protein structure subsequently leads to reprotonation followed by reisomerization of 13-cis retinal back to all-trans, and a return of the protein to its resting conformation. Transitions between intermediate states vary in rate from nanosecond to millisecond time scales, and these rates show marked temperature dependence.

High-resolution x-ray diffraction experiments on microcrystals of bR (Fig.1) have recently become possible, through the discovery by Landau and Rosenbusch that bR can be crystallized from the bi-continuous lipid-water gel formed by mono-olein. Consequently, the visualization of the precise structural changes responsible for the conversion of light energy into the proton-motive force is within reach. Members of our group have already collected a complete data set from microcrystals of bR in the resting state, and have begun collection of diffraction data from photocycle intermediates.

It is critical to our goal of obtaining an atomic model of bR in the M state that we confirm that we can trap the desired structural intermediate in the protein microcrystals. Towards this end we have undertaken to use ALS Beamline 1.4.3 to perform FTIR spectroscopy on individual microcrystals under conditions likely to thermally trap bR in the M state.

ACCOMPLISHMENTS

1) Mapping the positions of individual microcrystals -
The IR beam emanating from Beamline 1.4.3 is very bright, and can be focused to a diameter of as little as 10 microns, which is ideal for recording IR spectra from microcrystals whose diameter may not exceed 50 microns. Such a highly collimated beam does, however, require great precision in positioning the crystals that are to be its targets. In-house software has allowed us to easily align the IR beam with a target crystal by displaying spectral properties on a 2-dimensional grid (Fig. 2) whose coordinates correspond to the position of the microscope stage.

2) Recording the IR spectra of individual microcrystals -

Once a microcrystal of bR has been located with respect to the IR beam, we can collect spectra from the crystal itself and from the surrounding mono-olein gel. As expected, peaks signifying the presence of amide bonds appear in the former but not in the latter (Fig. 3).

3) Recording IR spectra at low temperature -
The lifetime of the M state is ~5 ms at ambient temperature, but bR persists in this state for minutes at 220-240K. In order to facilitate accurate cooling of our specimens the ALS purchased a state-of-the-art cryo-chamber that fits on the stage of the microscope through which the crystals are viewed. We have determined that IR spectra of bR microcrystals recorded at temperatures between 298K and 230K are essentially indistinguishable in the region of interest.

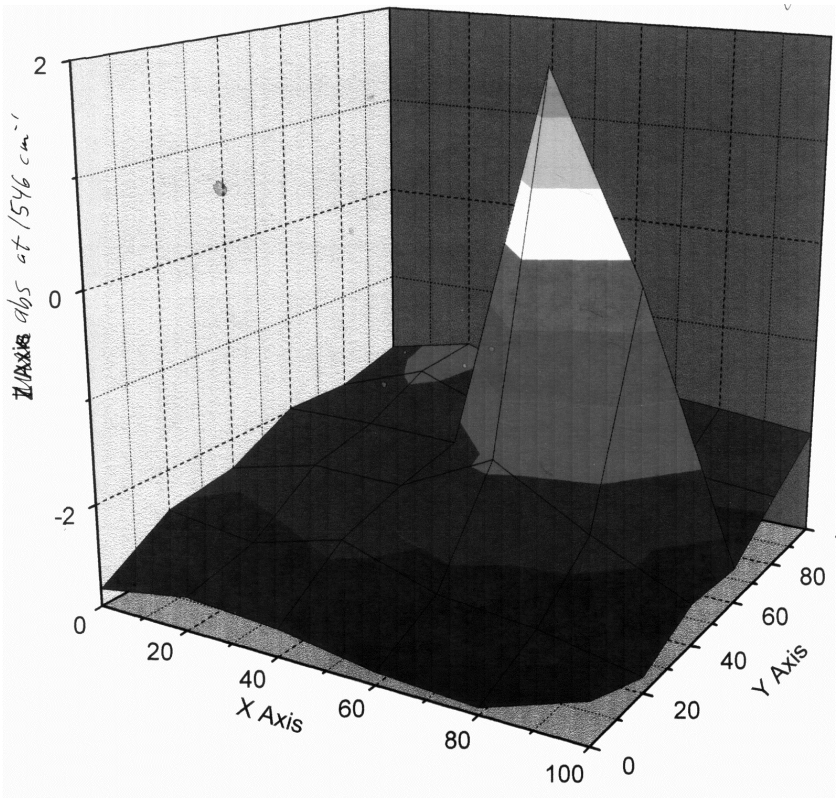
GOALS

1) Trapping bR microcrystals in the M state -
We are currently working to demonstrate the transformation of bR microcrystals from the resting state to the M state upon illumination at ~240K. An important step towards this goal was the insertion of a removable thin disk of Si or Ge in the path of the IR beam to absorb contaminating visible light so that the microcrystals remain in the dark
as they are cooled.

2) Trapping bR microcrystals in the L state-
A future goal is to work out conditions for trapping an earlier intermediate, the L state, prior to collecting x-ray diffraction data.

FIGURE 1. Hexagonal microcrystals of bR surrounded by mono-olein observed on a gold slide. This image was taken using the microscope at Beamline 1.4.3.

FIG.URE 2. Three-dimensional representation of absorp-tion at 1546 cm-1, which is diagnostic for protein, in a small area of the specimen. The peak corre-sponds to a bR microcrystal.



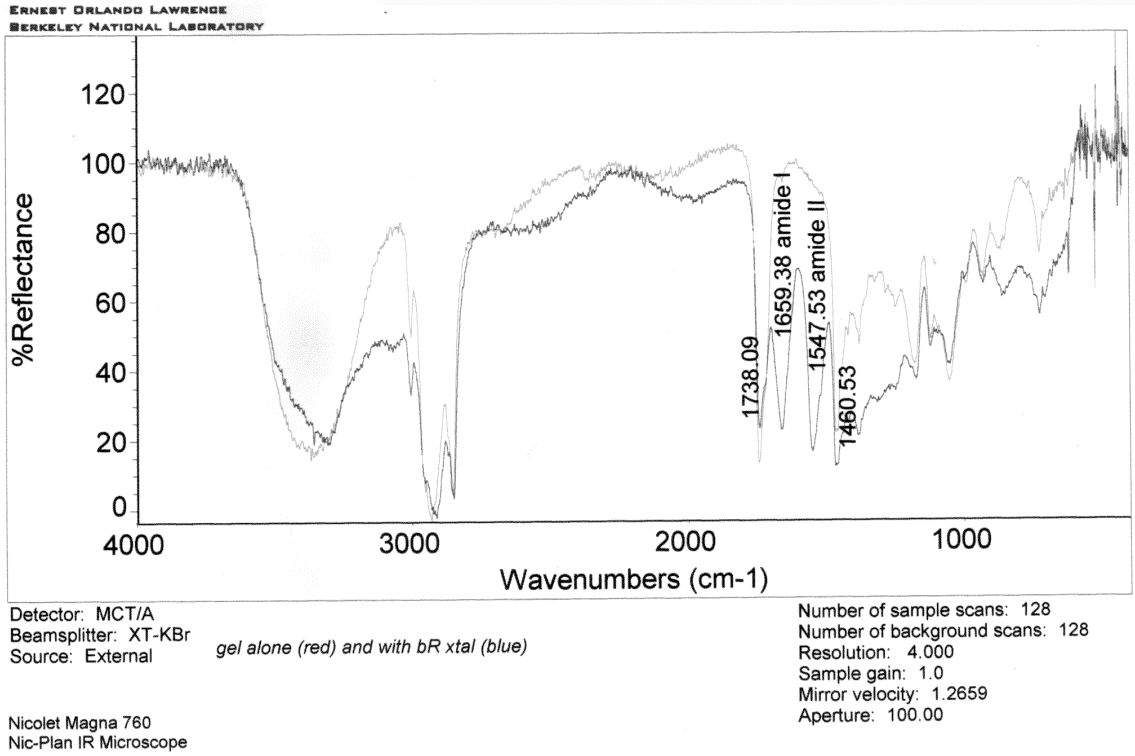


FIGURE 3. IR spectra of a bR microcrystal embedded in mono-olein (heavy line) and of a neighboring area containing only lipid (light line). The amide I and II bands arise from the peptide bonds of bR.